of the channel's opening transition without inhibiting voltage-sensor activation, thus partially uncoupling voltage-sensor activation from channel gating. Electromechanical coupling between the voltage-sensing domain (VSD) and the pore of Kv channels has been shown to depend on specific interactions between the S4-S5 linker and the carboxyl-terminal portion of the S6 segment. Here, we show that a single glycine to valine mutation in the S4-S5 linker of the Kv2.1 channel causes a large energetic destabilization of the channel's open state. Moreover, the G317V mutation also interferes with the channel's inhibition by CORM-2, suggesting that there is a cavity at the interphase between the VSD and the pore of the channel, including the S4-S5 linker, and that it is part of the CORM-2 binding site in the channel. These data are consistent with the mechanism of channel inhibition by this compound. Supported by Instituto de Ciencia y Tecnología del Distrito Federal, Grant PIFUTP09-262 to L.D.I.

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Intracellular Ions Impede Voltage Sensor Return in Kv1.2 Channels

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Voltage sensing in Kv channels originates from the coupling of movement between the charged S4 segment and the activation gate at the cytoplasmic region of the pore domain. The voltage sensor moves prior to the pore opening and the pore must shut before the voltage sensor returns to its resting state. However, gating current recordings from Kv channels indicate that frequently voltage sensors return more slowly after depolarisations that populate open states, indicating that the open pore exerts a resistance to S4 return. This process of pore closure therefore intrinsically regulates the deactivation kinetics of Kv channels. We observed slow voltage sensor return (Ig_{OFF}) in WT-Kv1.2 channels under non-permeant ionic conditions after depolarisations to voltages that caused channel openings. Using TEA^+ and NMG^+ internal solutions resulted in a slower Ig_{OFF} than internal Cs^+, suggesting that the intracellular ionic composition was modulating Ig_{OFF}. A mutation in the pore lining S6 segment to enlarge the inner cavity (Kv1.2-I402C) removed the slowing of Ig_{OFF} in the presence of internal NMG^+, suggesting that NMG^+ interacted within the inner cavity of the WT channel to prevent pore closure through a 'foot in the door' mechanism. Gating currents of a non-conducting, P-type inactivated channel (Kv1.2-W366F, V381T), in the presence of intracellular K^+ ions also displayed a slowing of Ig_{OFF} after depolarisations that would open the channel pore. These results suggest that internal K^+ ions bound in the inner cavity can also slow activation gate closure. We propose that internal ions in the cavity of Kv1.2 allosterically regulate the voltage sensor deactivation kinetics by preventing pore closure and thus rate limiting the return of voltage sensors.

2705-Pos Board B475

Voltage-Dependent Gating of the K+ Channel KvLm Explored through Heterotetramers

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Voltage-gated K+ (Kv) channels are tetrameric assemblies in which each subunit is modular in design and consists of a voltage-sensor and a pore. KvLm, the voltage-gated K+ channel from Listeria monocytogenes differs from other Kv channels in that its voltage-sensor contains only three out of the eight charged residues implicated in voltage-gating. Here we ask how many sensors are required to produce a functional Kv channel by investigating heterotetramers comprising combinations of KvLm full-length (FL) and its sensorless pore-module (PM). Accordingly, we studied the voltage-dependent properties of KvLm channels with 0, 1, 2, 3 and 4 voltage sensors. We show that KvLm heterotetramers produced by cell-free expression yield functional channels after reconstitution in droplet interface bilayers. Further, we demonstrate that three voltage-sensors are sufficient to recapitulate the voltage-dependent activation features of wild-type KvLm, whereas deletion of two or more sensors severely suppresses the voltage-dependent closure and activation of the assembled channel. The current-voltage relationship of all heteromers remains similar. We also demonstrate that all four voltage-sensors are required to keep the channel closed at hyperpolarizing potentials, and that deletion of all four sensors results in a pore-only assembly, which retains limited voltage-dependence.

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Allosteric Stabilization of Fully Resting Voltage Sensors by a Tarantula Toxin

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The mechanism of a tarantula toxin's action on the voltage gating of a K channel was investigated. An oxidation-resistant variant of guangxitoxin-1E (GxTX) with methionine 35 replaced by norleucine, was synthesized and found to retain biological activity. When applied to voltage-clamped CHO-K1 cells expressing rat Kv2.1, this GxTX was found to shift channel opening to more positive voltages. In response to short stimulating voltage steps, the voltage shift of conductance saturated at micromolar GxTX concentrations. Prolonged or repetitive pulses to positive potentials ejected GxTX from Kv2.1 channels, revealing the decreased affinity of GxTX for activated voltage sensors. GxTX positively shifted Kv2.1 gating currents, and prevented outward gating charge movement at negative voltages. The modulation of gating charge movement indicates that GxTX stabilizes gating charges in their most internal conformation. Single Kv2.1 channels with GxTX bound exhibited a similar unitary conductance as without tarantula toxin, but had an increased latency to first opening in response to positive voltage steps. A diminished mean open time in the presence of GxTX confirms that channel openings occur with toxin bound and suggests a mechanism for the toxin-induced decrease in peak conductance of macroscopic currents. A simple allosteric model was developed where GxTX stabilizes the earliest resting state of voltage sensors. In this model, GxTX binds activated voltage sensors with decreased affinity, and exerts only a feeble destabilizing influence on the dominant open state. The difference in binding affinity between resting and activated voltage sensors suggests potential for development of GxTX as a probe of voltage sensor conformation in living cells.

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Activation Gate Opening Precedes Slow Inactivation in Shaker K+ Channels

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In the absence of N-type inactivation Shaker potassium channels display slow (C-type) inactivation. Functional and structural studies indicated that channel opening precedes slow inactivation in voltage-gated and KcsA K+ channels, whereas others argued for slow inactivation from the closed sate of the channel as well. None of the previous studies correlated the opening of the of the activation gate (A-gate), formed by the bundle crossing of the S6 segments, with the development of inactivation, which is associated with the structural rearrangement in the selectivity filter. These two gates are coupled and thus, the current experiments addressed the hypothesis that opening of the activation gate must precede slow inactivation.

To address this hypothesis we compared the voltage dependence of A-gate opening and that of the development of inactivation in T449A/V474C Shaker-IR channels. Opening of the A-gate was monitored by the accessibility of 474C to Cd2+ from the intracellular side. The membrane potential was changed repeatedly from a holding potential of −120 mV to test potentials ranging from −110 mV to −60 mV in the presence or absence of Cd2+.

Our results show that the function describing the voltage dependence of Cd2+ block is shifted toward the negative potentials compared to the voltage dependence of steady-state inactivation curve. This indicates that A-gate opening already occurs at such negative potentials where no inactivation can be detected. Furthermore, the curve representing Cd2+ block is also negative-shifted compared to the voltage dependence of steady-state activation (G-V) curve. This suggests that even at fairly negative holding potentials, at which no macroscopic current can be detected, rare channel openings occur yielding access to the channel cavity. Based on these results we suggest that A-gate opening always precedes structural changes associated with slow inactivation.

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Gating Properties and Voltage Sensing in Kv1.2

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Kv1.2 is a voltage gated potassium channel whose crystal structure has been solved. There is markedly little electrophysiological information about it, which limits the vast potential usefulness of the structure. In an effort to deepen our functional understanding of the Kv1.2 channel, we set out to characterize its properties through recordings of macroscopic and single ionic and gating currents and develop models for activation based on established models for other Shaker channels. Preliminary data indicate that even though Shaker and Kv1.2 are closely related, subtle differences exist in their mechanisms of voltage sensing. As an example of these differences, limiting slope analysis reveals that the apparent charge coupled to gating of Kv1.2 is only 10 e0 compared to Shaker's 13 e0. Also, substitution of Kv1.2's tryptophan at position 366, the analogue of W434 in Shaker, for the other two aromatic residues does not abolish conduction as in Shaker. Our macroscopic data are best fitted by a 32 state model involving three independent transitions for each of the subunits and two final, voltage independent, concerted transitions to the open state.